Kong, China 4 Department of Genetics, UTMDACC, Houston, TX, USA LIM-homeodomain genes Lhx1 and Lhx5 are required for the differentiation of Purkinje cells (PC), one of the principal cerebellar neurons which play a vital role in controlling motor coordination and balance. Interestingly, high levels of Lhx1/5 expression persist even after PC differentiation. Hence, there may be additional roles for these two genes during postnatal PC development. To address this question, an Lhx1/5 double conditional knockout (DKO) mutant was generated and crossed with a PC-specific Pcp2-IRES-Cre mouse, thus inactivating the two genes beginning at postnatal day 2. The DKO mutants show initially modest but noticeable ataxic locomotion at around two weeks after birth. However at 8 weeks, the mutants displayed severe deficits in motor coordination and body balance but not the control animals with one functional copy of either gene. Although the general cytoarchitectural lamination of cerebellar cortex was maintained, thinner and less extensive PC dendrites were observed in the mutants. Such defective dendritic arborization suggests a possible underlying mechanism leading to the abnormal behavioral phenotypes. Thus, Lhx1/5 are required and function redundantly in postnatal development of PC. Further studies are underway to dissect the molecular consequences of Lhx1/5 ablation in Purkinje cells.

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Program/Abstract # 174
Cellular compartments and differential cell behaviors underlie formation of the distinct foliation pattern of the mouse cerebellum

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The mouse cerebellum (Cb) has a complex three-dimensional (3D) organization contrasting with its relatively simple layered cytoarchitecture. The Cb is divided along its antero-posterior axis into folia, each separated by fissures; the pattern of foliation varies along the medio-lateral axis defining a medial vermis and two lateral hemispheres. Each folium has a distinct shape. We are asking how the diversity of folia shapes is generated during Cb development. We have focused on granule cell (gcs) since their proliferation is critical for Cb foliation. By carrying out a genetic clonal analysis of gcs, our preliminary results indicate that the base of each fissure acts as a boundary restricting gc dispersion between folia. Thus, two adjacent fissures could define a developmental compartment that in turn facilitates differential regulation of cell behaviors in each folium. Using genetic inducible fate mapping, we found that the kinetics of gc production differ in subsets of folia. Furthermore, in a Cb foliation mutant, the fissures form at different times and in a different order compared to wild type mice, and the kinetics of gc production are altered. These results suggest that the differential regulation of cell behaviors in Cb compartments could account for the acquisition of distinct regional foliation patterns in the Cb. In addition, in many Cb foliation mutants, the definition of compartments and their respective kinetics of gc production is likely altered, leading to an overall different 3D organization compared to wild type mice.

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Program/Abstract # 175
ENU mutagenesis identifies novel genes required for forebrain development

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We have performed an ENU mutagenesis screen in the mouse specifically focusing on mutations affecting neurodevelopment and have identified eight mutations. Here we summarize our findings and discuss our current studies on two of these novel genes affecting cortical development. The most remarkable phenotype uncovered to date is the rudolph mutation, which has severe developmental defects in both the CNS and appendicular skeleton. The organization of the neocortex is profoundly disrupted and contains clustered cell bodies that appear to be neurogenic foci. The causal gene is the cholesterol biosynthesis enzyme Hsd17b7, which is notable given the recent implication of a role for oxysterols in mediating intracellular components of Hedgehog signaling. We see decreased induction of known Sonic hedgehog (Shh) target genes both in vivo and in vitro, revealing a requirement for embryonic cholesterol metabolism in both CNS development and normal Shh signaling. We also have discovered a line we call brain dimple (brdp), which carries a mutation in beta tubulin, 2b (Tubb2b). This mutant has an extreme reduction in cerebral cortex tissue and mutations in TUBB2B have recently been identified in human patients with asymmetrical polymicrogyria. The brdp phenotype emerges after the onset of mouse neurogenesis (E11.5) and we are currently studying the mechanism of Tubb2b function in the cortex. Brdp/+ adult mice have a hyperactivity phenotype and we show they have cortical hypocellularity and defects in cortical lamination.

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Program/Abstract # 176
Cranial vessel formation in the developing zebrafish

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Brain vascular disease is a topic of intensive study. Understanding the developmental biology of cranial vessel formation is important for understanding both development of the central nervous system and cerebrovascular pathologies such as stroke. The zebrafish has proven itself a useful model for elucidating the molecular basis of human disease. A detailed study of the vascular anatomy of the developing zebrafish embryo has been prepared, revealing that the zebrafish has a vascular formation plan that is well conserved compared to other vertebrates, including humans. We are using the zebrafish to better understand the mechanisms underlying cranial vascular development by (i) carrying out a precise anatomical description of the formation process, (ii) investigating the molecular mechanisms guiding vascular patterning and assembly in the brain, and (iii) using genetic screens to identify new genes important for brain vessel formation. We have examined hindbrain vascular development using two-photon time-lapse imaging of transgenic zebrafish expressing green fluorescent protein (GFP) in vascular endothelial cells. We have found that one of the most important brain arteries, the basilar artery, is formed by sprouting of angioblasts from pre-existing primitive veins. Once the sprouts coalesce at the midline, their connections to the primitive veins are lost, making this a unique blend of vasculogenesis- and angiogenesis-like vessel assembly processes. By examining potential guidance cues expressed in the hindbrain, we have also discovered that chemokine signaling is required to direct basilar artery assembly. We will report on these and other novel molecular mechanisms directing the assembly of cranial vascular networks.

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